Unraveling the impact of arsenic on the redox response of peanut plants inoculated with two different *Bradyrhizobium* sp. strains

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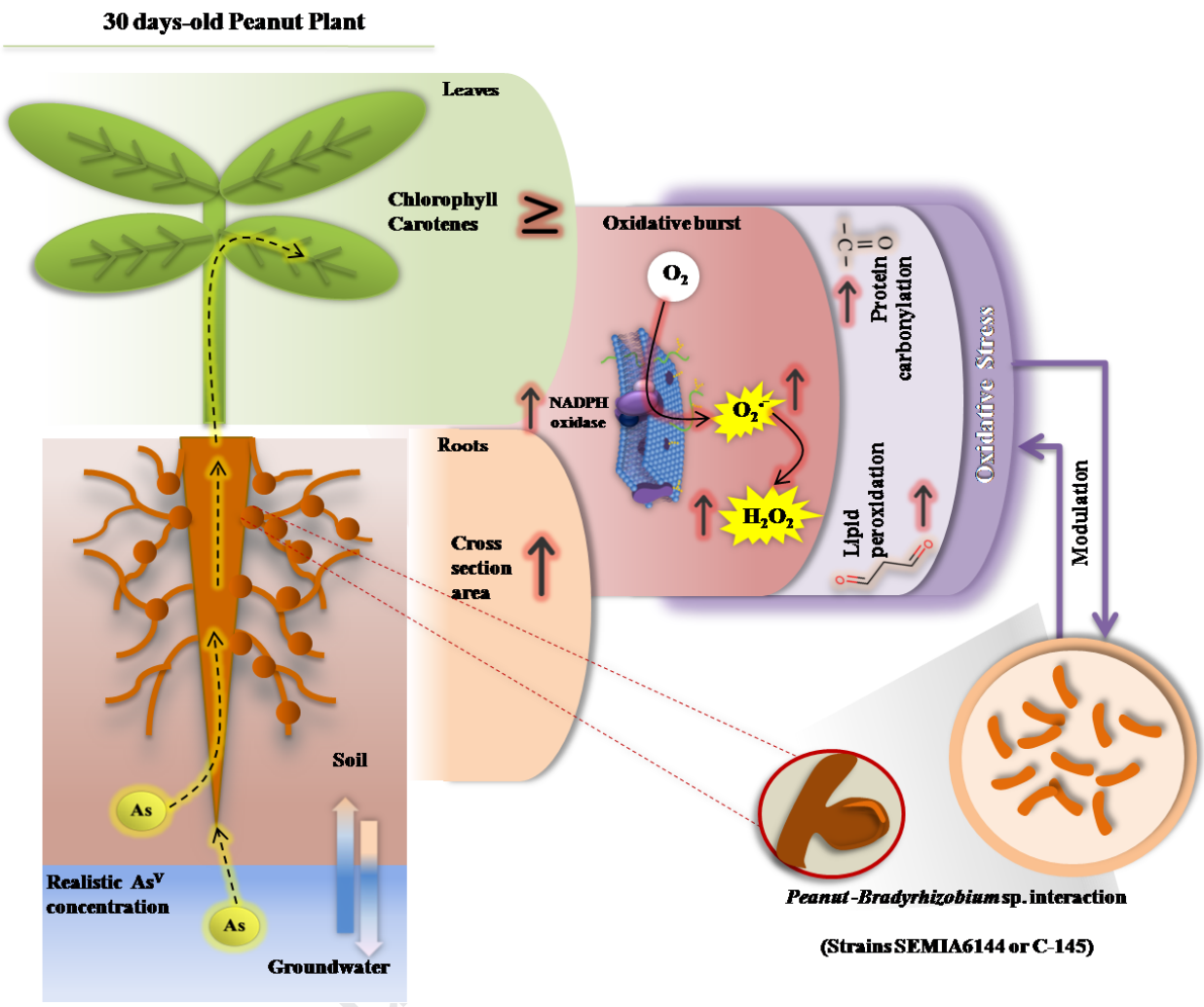
30 days-old Peanut Plant
Leaves
Chlorophyll
Carotenes
Oxidative burst
O_2
NADPH oxidase
$O_2^{\cdot-}$
H_2O_2
Protein carbonylation
Lipid peroxidation
Oxidative Stress
Modulation
Roots
Cross section area
Soil
As
As
Realistic As^V concentration
Groundwater
Peanut-Bradyrhizobium sp. interaction
(Strains SEMIA6144 or C-145)

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ABSTRACT

Arsenic (As) can be present naturally in groundwater from peanut fields, constituting a serious problem, as roots can accumulate and mobilize the metalloid to their edible parts. Understanding the redox changes in the legume exposed to As may help to detect potential risks to human health and recognize tolerance mechanisms. Thirty-days old peanut plants inoculated with *Bradyrhizobium* sp. strains (SEMIA6144 or C-145) were exposed to a realistic arsenate concentration, in order to unravel the redox response and characterize the oxidative stress indexes. Thus, root anatomy, reactive oxygen species detection by fluorescence microscopy and, ROS histochemical staining along with the NADPH oxidase activity were analyzed. Besides, photosynthetic pigments and damage to lipids and proteins were determined as oxidative stress indicators.

Results showed that at 3 µM As^V, the cross-section areas of peanut roots were augmented; NADPH oxidase activity was significantly increased and $O_2^{\cdot -}$ and H_2O_2 accumulated in leaves and roots. Likewise, an increase in the lipid peroxidation and protein carbonyls was also observed throughout the plant regardless the inoculated strain, while chlorophylls and carotenes were increased only in those inoculated with *Bradyrhizobium* sp. C-145. Interestingly, the oxidative burst, mainly induced by the NADPH oxidase activity, and the consequent oxidative stress was strain-dependent and organ-differential. Additionally, As modifies the root anatomy, acting as a possibly first defense mechanism against the metalloid entry. All these findings allowed us to conclude that the redox response of peanut is conditioned by the rhizobial strain, which contributes to the importance of effectively formulating bioinoculants for this crop.

Keywords: Metalloid • *Arachis hypogaea* L. • Rhizobia • Reactive Oxygen Species • Oxidative stress

Abbreviations

As: Arsenic

As^V: Arsenate

ROS: Reactive oxygen species

NADPH: Nicotinamide adenine dinucleotide phosphate

$O_2^{\cdot -}$: Superoxide anion radical

H_2O_2: Hydrogen peroxide

$^{\cdot}OH$: Hydroxyl radical

BNF: Biological nitrogen fixation

PO_4^{3-}: Phosphate

DHE: Dihydroethidium

DCF-DA: 2´-7´-dichlorofluorescein diacetate

TMP: Tetramethyl piperidinooxy

DAB: 3, 3-diaminobenzidine

NBT: Nitroblue tetrazolium

TBA: Thiobarbituric acid

TBARS: Thiobarbituric-reactive substances

1. Introduction

Peanut (*Arachis hypogaea* L.) is a legume plant, originally from South America. The fruit is a pod with one to five seeds that develops underground (Boote *et al*., 1982). This legume is considered the 13th most important food crop and the 4th major source of edible oil. Argentina constitutes one of the top peanut exporting countries, being Córdoba province the main producer (Agriexchange, 2017). Generally, all peanut parts are used for some purposes as primary crop or as a source of several food products (Argentine Peanut Chamber, 2012; Pedelini, 2014). This legume can engage in a symbiotic interaction with rhizobia, resulting in the formation of specialized root nodules able to fix atmospheric nitrogen. The legume-rhizobia interaction is mediated by a complex molecular signal exchange, where recognition of different bacterial determinants activates the nodulation program in the plant (Ibañez *et al*., 2016). However, different stresses such as heavy metal and metalloid presence in the environment could negatively affect this interaction and contaminate the edible seed (Bianucci *et al*., 2020). In this regard, a metalloid that has gained special interest in the past years is arsenic (As) (Smedley and Kinniburgh, 2002). This metalloid is naturally found in soil and water and, several peanut producing areas in Córdoba, present groundwater containing high arsenic concentrations, coming from natural sedimentary depositions (Cabrera *et al*., 2005; Francisca *et al*., 2006). In some regions, As levels reach up to 24 µM, a concentration that exceeds the maximum allowed for drinking water, which is 0.1 µM (Cabrera *et al*., 2005; FAOSTAT, 2016). The main problem lies on the fact that this water can be directly absorbed by plants or be used for artificial irrigation of crops constituting the first stage of As distribution in the trophic chain (Smedley and Kinniburgh, 2002; Bustingorri and Lavado, 2014). Thus, peanut contamination could constitute a serious agronomic and public health problem.

Arsenic occurs both in organic and inorganic forms, being the latter the most toxic (Zhao *et al*., 2009). The inorganic form arsenate [As^V as $H_2AsO_4^-$ or $HAsO_4^{2-}$] predominates in aerobic environments as it occurs in extensive areas of Cordoba´s groundwater (Blarasin *et al*., 2014). Arsenate (As^V) is an analog of phosphate (PO_4^{3-}) and it can be absorbed by cells via phosphate (PO_4^{3-}) transporters (Tripathi *et al*., 2007; Catarecha *et al*., 2007; Wang *et al*., 2018) inducing several alterations to essential cellular processes (Bianucci *et al*., 2020). As an evolutionary response, plants have developed a range of strategies to counteract As toxicity. In this

sense, As^{V} is rapidly transformed by different arsenate reductase enzymes to arsenite [As^{III} as $H_3AsO_3^0$ or $H_2AsO_3^-$] (Sánchez-Bermejo *et al*., 2014). Even though As is not a redox metal, there is significant evidence showing that plant exposure to inorganic As results in an oxidative burst, evidenced by the over-production and/or accumulation of reactive oxygen species (ROS) that can oxidize important biomolecules (lipids, proteins and nucleic acids) leading to oxidative stress (Talukdar *et al*., 2013; Hernández *et al*., 2015; Bianucci *et al*. 2017, 2018; 2019). ROS are the product of the normal aerobic metabolism; these are oxygen reduced forms and result from the excitation of oxygen to singlet oxygen ($^1O_2$) or the gain of one, two or three electrons to form superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) or hydroxyl radical ($^{\bullet}OH$), respectively (Mittler, 2017). Another important source of ROS is the NADPH oxidase enzyme, associated with the plasma membrane that has a low redox potential to produce $O_2^{\cdot -}$ (Torres and Dangl, 2005; Mittler *et al*., 2011). The dual role of ROS, as toxic or signal molecules, is site-specific, dose-dependent and, in cells, this is determined by the quotient between the rates of generation and degradation, carried out by the antioxidant defense system (Foyer *et al*., 2011; Mittler, 2017). Although it is known that As reduces growth and productivity due to a plethora of morphological, physiological, biochemical and molecular alterations (Begum *et al*., 2016; Singh *et al*., 2017; Shahid *et al*., 2017; Bianucci *et al*., 2017; 2018; 2019; Peralta *et al*., 2019), the over-production and/or accumulation of ROS that leads to oxidative stress, results in the most detrimental biochemical effect at the sub-cellular level.

Many researches have tested metal(loid)s impact on legume plants, however, most of them were performed using metalloid concentrations that exceed natural As levels, making it difficult to extrapolate the findings to field conditions (Sharma, 2012a ; Gupta *et al*., 2013; Ghosh *et al*., 2016; Upadhyay *et al*., 2018). In addition, the use of rhizobia to reduce metal(loid)s toxicity is a very important strategy that has been taken into consideration in the past years due to the ability of these microorganisms to promote plant growth and also reduce metal(loid) toxicity (Ma *et al*., 2016). The capacity of these bacteria to counteract As is related to their own metal(oid) resistance system that allows for chemical and physical transformation of the contaminant element, being able to chelate, precipitate or induce metal(oid)s sorption (Delgadillo *et al*., 2015; Yang and Rosen, 2016; Fagorzi *et al*., 2018). In the symbiosis with legume plants, which are generally non-hyperaccumulating species, the key contribution of rhizobia besides plant promotion, is to increase the phytostabilization process of the metal(oid)s compared to non-inoculated plants (Dary *et al*., 2010; Bolan *et*

al., 2011; Kong *et al*., 2017; Kidd *et al*., 2017; Bianucci *et al*., 2020).

Until now, information regarding oxidative modifications induced by arsenic on legume plants inoculated with different rhizobial strains is scarce. Previous results obtained in our laboratory have shown that a realistic concentration of As^V had a negative impact on peanut growth and that, the inoculation with *Bradyrhizobium* sp. strains (SEMIA6144 and C-145, previously characterized by its differential tolerance to the metalloid, Bianucci *et al*. (2016)) conditioned the growth variables and metalloid translocation to the edible parts (Peralta *et al*., 2019). Based on these facts, our experiments were performed in order to unravel the redox response of peanut plants exposed to a natural concentration of As, when inoculated with a *Bradyrhizobium* strain, sensitive or tolerant to the metalloid. This study will allow us to reveal key aspects of oxidative mechanisms triggered in the peanut-rhizobia symbiotic interaction exposed to a realistic concentration of the metalloid.

2. Material and methods

2.1. Bacterial strains

Bradyrhizobium sp. SEMIA6144 and *Bradyrhizobium* sp. C-145 strains were obtained from MIRCEN (Brazil) and INTA (Argentina), respectively. Bacterial cultures were grown Yeast Extract Mannitol broth (YEM) (Vincent, 1970) medium and incubated at 28 ºC on a gyratory shaker at 150 rpm until they reached an OD_{620nm} corresponding to 10^8CFU mL^{-1} (colony forming unit per milliliter). The number of viable cells was determined following the method described by Somasegaran and Hoben (1994).

2.2. Plant material and experimental design

Peanut seeds cv. Granoleico (El Carmen S.A; General Deheza, Córdoba, Argentina) were surface disinfected following the method described by Vincent (1970). Then, they were germinated at 28ºC in Petri dishes on a layer of Whatman number 01 filter paper and moistened cotton, until the radicle reached 2-3 cm. Seedlings were transferred to a Leonard Jar system (Figure S1) as previously described in Peralta *et al*. (2019). Hoagland´s nutrient solution (Hoagland and Arnon, 1950) devoid of As^V (control) or containing 3 µM As^V, the average concentration of the metalloid found in groundwater of Córdoba (Cabrera *et al.,* 2005) was used. The As^V was supplied as $Na_2HAsO_4 \cdot 7H_2O$ and the liquid level in the lower cup remained constant throughout

the entire experience. At 7 days post-emergence, peanut seedlings were inoculated with *Bradyrhizobium* sp. SEMIA6144 or *Bradyrhizobium* sp. C-145 (sensitive and tolerant to As, respectively). Plants were grown in a controlled environment chamber (light intensity of 200 µmol m^{-2} s^{-1}, 16 h day/8 h night cycle, a constant temperature of 28°C and a relative humidity of 50%) for 30 days post-inoculation. At harvest, leaves and roots were used for different analysis.

2.3 Root anatomy

To examine As-induced anatomical and histological changes, peanut fresh main root was cut into 5 mm length portions, at 1 cm from the root tip as described in Travaglia *et al*. (2012). Root sections were processed using microtechnical methods following Johansen's recommendations (1940), which involved dehydration, infiltration and paraffin embedding of tissues, rotary microtome cutting and staining. A standard Zeiss Model 16 microscope was used to assess the histological preparations and, photomicrographs were taken using a Zeiss Axiophot microscope with an AxioVision 4.3 image capture and digitalization piece of equipment and an AxioCamHRc camera.

2.4 Indicators of ROS production

2.4.1 Determination of NADPH oxidase activity and hydrogen peroxide (H_2O_2)

NADPH oxidase activity was determined spectrophotometrically by nitroblue tetrazolium (NBT) reduction at 560 nm (Sagi and Fluhr, 2001). Fresh plant material (roots and leaves) was homogenized according to Sobrino-Plata *et al*. (2009) procedure. The activity was measured in 1 mL reaction buffer containing: 0.02 mg protein extract, 0.5 mg mL^{-1} NBT, 0.2 mM NADPH, 4 mM $CaCl_2$ and 0.2 mM $MgCl_2$. One unit of NADPH oxidase was defined as the quantity of enzyme needed to reduced 1 µmoL NADPH min^{-1}.

Hydrogen peroxide concentration was determined by spectrophotometry after reaction of roots and leaves extracts with potassium iodide (KI) following the procedure described by Alexieva *et al*. (2001) with modifications. Plant material (100 mg) was homogenized in 0.1% (w/v) of trichloroacetic acid (TCA) and centrifuged at 10,000 g. Then, an aliquot of the supernatant was mixed with a reaction buffer containing 100 mM potassium phosphate, pH 6.8 and KI 1M. The reaction was carried out 1 h in dark. The amount of H_2O_2 was calculated using a standard calibration curve prepared with known H_2O_2 concentrations.

168

169 **2.4.2 ROS detection by fluorescence microscopy *in situ***

170 For the histological detection of ROS in peanut roots, fresh segments of the main root were cut approximately
171 2 cm from the root apex.

172 Superoxide anion radical ($O_2^{\cdot-}$) was imaged by incubating root segments with 10 µM dihydroethidium (DHE)
173 (Fluka Biochemika, Buchs, Switetzerland) prepared in 10 mM Tris-HCl, pH 7.4 for 30 min, as indicated by
174 Sandalio *et al*. (2008) and observed in a stereomicroscope (excitation at 488 nm, emission at 520 nm). The use
175 of DHE has been described as a specific fluorescent probe for $O_2^{\cdot-}$ (Fink *et al*., 2004). Specificity of the
176 reaction was carried out by using the superoxide specific sequester tetramethyl piperidinooxy (TMP; 1mM).
177 Hydrogen peroxide (H_2O_2) was histologically detected by incubating the root with 25 µM 2´-7´-
178 dichlorofluorescein diacetato (DCF-DA) (Calbiochem, San Diego, CA, USA) prepared in 10 mM Tris-HCl
179 buffer, pH 7.4 for 30 min as described by Rodríguez-Serrano *et al*. (2006). Samples were then observed in a
180 stereomicroscope (excitation at 485 nm, emission at 530 nm). Several studies have demonstrated that DCF can
181 also react with other peroxides, mainly hydroperoxides in the presence of peroxidases (Tarpey *et al*., 2004).
182 Accordingly, as negative control, root pieces were pre-incubated in darkness for 1 h at 25ºC, with 1 mM
183 ascorbate (H_2O_2 scavenger). Chemicals were prepared in the same buffer used for the fluorescent probes
184 (Sandalio *et al*., 2008).

185

186 **2.4.3 ROS histochemical detection in leaves**

187 *In vivo* localization of H_2O_2 was performed by incubating freshly vegetal material in 1 mg mL^{-1} 3,3-
188 diaminobenzidine (DAB) for 8 h (Orozco-Cárdenas and Ryan, 1999). Superoxide anion ($O_2^{\cdot-}$) was visually
189 detected incubating freshly vegetal material in 1 mM NBT, prepared in 10 mM sodium citrate buffer pH 6, for
190 8 h, following the procedure described by Frahry and Schopfer (2001). Finally, the photographs were taken
191 using a Dell® Scanner.

192

193 **2.5 Oxidative stress indexes**

194 Photosynthetic pigments were extracted from 100 mg of fresh leaves in ethanol 80%. After incubation in a
195 thermostated bath at 100 ºC for 15 min, the absorbance of the solution was measured at 662 nm for

196 chlorophyll *a*, 645 nm for chlorophyll *b* and 470 nm for carotenoids. Chlorophyll and carotenoides content
197 was calculated using the formula given by Vernon and Mac Kinney (modified by Mac Kinney, 1938):
198 Chlorophyll *a*= 11.63 (DO_{665nm}) – 2.39 (DO_{650nm}); Chlorophyll *b*= 20.11 (DO_{650nm}) – 5.18 (DO_{665nm}); Total
199 chlorophyll= Chlorophyll *a* + Chlorophyll *b*; Carotenoids= 0.02 (DO_{450nm}).
200 Oxidative damage was analyzed in roots and leaves through the quantification of lipid peroxidation and
201 protein carbonylation. Lipid peroxidation was evaluated by determining the concentration of thiobarbituric-
202 reactive substances (TBARS) as described by Heath and Packer (1968) with modifications. Plant material
203 (100 mg) was homogenized in 0.1% (w/v) of TCA and mixed with 0.5% (w/v) of TBA. The extract was
204 heated for 25 min at 95ºC and the reaction was stopped on ice. Then, the samples were centrifuged at 6,200 g
205 for 6 min. TBARS were quantified by measuring absorbance at 535 nm and corrected for non-specific
206 turbidity by subtracting the absorbance at 600 nm, using a UV-visible light spectrophotometer (Spectronic®
207 Genesys 2, USA). Oxidation of proteins was analyzed by quantification of the reactive carbonyl groups to 2,4-
208 dinitrophenylhydrazine (DNPH) to form hydrazones (Levine *et al.*, 1990). Plant material (250 mg) was
209 homogenized in buffer containing 100 mM potassium phosphate, pH 7, 0.1% (v/v) Triton X-100, 1 mM
210 Na_2EDTA and 0.5 mg mL^{-1} leupeptine. Then, the samples were centrifuged at 12,000 g for 15 min at 4ºC. The
211 supernatant was extracted and 1% (w/v) streptomycin sulfate was added. After a second centrifugation, the
212 supernatant was divided in two aliquots. To determine carbonyls, the supernatant was mixed with 20 mM
213 DNPH prepared in 2N HCl. To determine proteins, the supernatant was mixed with 2N HCl. Both aliquots
214 were kept in agitation for 1 h and then, 10% (w/v) TCA was added. After centrifugation (6,000 g, 15 min at
215 4ºC), the pellet was washed three times with ethanol: ethyl acetate (1:1). The precipitated proteins were
216 solubilized in 6 M guanidine-HCl, pH 4.5 for 30 min under agitation. Finally, a centrifugation at 6,000 g for 2
217 min was made. The amount of protein carbonyls was calculated using extinction coefficient 22,000 mol^{-1} cm^{-1}
218 and values were expressed in terms of nmol mg^{-1} protein.
219
220 **2.6 Data analysis**
221 Experiments were performed in a completely randomized design with five replicates and three independent
222 experiments. The data were analyzed using the *InfoStat* program (Di Rienzo *et al.*, 2018). ANOVA was
223 performed and significant differences between treatments were calculated by Duncan´s test at $P < 0.05$. Prior

to the test of significance, the normality and homogeneity of variance were verified using the modified Shapiro-Wilk and Levene tests, respectively. All graphs were created by *Origin Pro* 2018b (OriginLab, USA).

3. Results

3.1 Effect of arsenate on root anatomy

Figure 1 shows that in all treatments, regardless of the inoculated strain, the three tissue systems dermal, fundamental and vascular were recognized. The last two were distributed forming two well-differentiated zones, the cortex and the central cylinder. The uniestratified epidermis and the strata adjacent, formed by isodiametric cells of parenchymal characteristics, were also identified. Roots were classified as triarch type, determined by the projections number of the protoxylem inside the vascular cylinder. Interestingly, the addition of As^V significantly increased the total area of peanut roots by approximately 3-fold higher than in control condition, irrespective of the bacterial strain used as inoculant. At the same condition, the vascular cylinder area showed a similar behavior (Table 1).

3.2 Photosynthetic pigments content as a stress indicator

The addition of As^V did not modify the photosynthetic pigment content of the peanut- *Bradyrhizobium* sp. SEMIA6144 symbiotic interaction respect to control treatment. However, in peanut plants inoculated with the tolerant strain *Bradyrhizobium* sp. C-145, a significant increase of chlorophyll *a* and *b* (which enhanced total chlorophyll) and carotenoids content were detected (Table 2).

3.3 ROS production and oxidative damage under arsenate conditions

3.3.1 NADPH oxidase activity

Addition of As^V increased NADPH oxidase enzyme activity in peanut plants, regardless the inoculation condition and the organ analyzed. This augmentation was clearly exacerbated in roots of the symbiotic interaction established between peanut and the tolerant strain *Bradyrhizobium* sp. C-145 (8-fold respect to control condition) (Figure 2).

3.3.2 Histochemical detection and quantification of H_2O_2

Figure 3 shows H_2O_2 detection in roots and leaves of peanut plants. Peanut roots exposed to As^V showed a higher fluorescence emission compared to control condition, irrespective of the inoculated strain (Figure 3I). Similarly, DAB staining of peanut leaves revealed that, regardless of the strain used as inoculant, an increase in dark brown precipitates was observed when arsenate was added compared to control condition (Figure 3II). The H_2O_2 histochemical observation, in roots and leaves, was consistent with the quantitative analysis of this specie in which a significant increase was observed. The increase detected in roots and leaves roots of peanut inoculated with *Bradyrhizobium* sp. SEMIA6144 was significantly higher than that obtained when *Bradyrhizobium* sp. C-145 was added as inoculant (Figures 3III and 3IV).

3.3.3 *In vivo* histochemical detection of $O_2^{\cdot -}$ in peanut plants

Irrespective of the inoculated strain, a higher red fluorescence emission was observed in peanut roots exposed to As^V, compared to control treatment (Figure 4I). Likewise, the leaves of peanut plants grown under As exposure, showed an increase in blue precipitates, indicating $O_2^{\cdot -}$ presence (Figure 4II).

3.4 Oxidative damage in peanut plants

Arsenate caused a significant TBARS increase in leaves from plants inoculated with *Bradyrhizobium* sp. SEMIA6144 (Figure 5A). Besides, in peanut roots, the addition of the metalloid caused an increase on TBARS content when peanut was inoculated with either of the strains tested, compared to control treatment (Figure 5B). In addition, As effect was significantly higher in the symbiotic interaction between *Bradyrhizobium* sp.SEMIA6144 and peanut. Regarding oxidation of proteins, the results indicated that As^V triggered an increase on the content of carbonylated proteins, respect to plants devoid of As, irrespective of the inoculated strain and the evaluated tissue (Figure 6).

4. Discussion

The effects of arsenic on several plant species have been extensively studied (Souri *et al*., 2017; Abbas *et al*., 2018; Bianucci *et al*., 2017; 2018; 2019; 2020). However, the analysis of the impact of natural or realistic concentrations of the metalloid on agronomic crops in symbiosis with members of the edaphic microbiome is scarce. Plants resort to acclimatization strategies in response to abiotic environmental stress, some of it is

related to changes in physiological and biochemical processes, as well as morphological and developmental patterns (Schikora and Schmidt, 2001; Pasternak *et al*., 2005). One of the most common stress responses of plants to metalloid addition is the induction of changes in the anatomy of the root, since it is the main tissue that is in direct contact with the contaminant (Deng *et al*., 2019). In this regard, it was reported that the root of *Arachis hypogaea* L. plants, exposed to As^V (20 and 100 µM) showed an increase in cell wall thickness and the disintegration and rupture of epidermal and parenchymal cells of the cortex (Bianucci *et al*., 2017). In addition, *Phaseolus aureus* Roxb plants exposed to high As^V concentrations (10-50 µM) exhibited severe damage to root tissue, a reduction of root hairs and an alteration of the vascular tissue shape (Singh *et al*., 2007). According to our results, 3 µM As^V treatment increased the cross-section area of the main root of peanut plants due to an increase in the number of layers in the cortex zone. It is known that cells that form the cortex zone are relatively undifferentiated and the number of layers in this root zone is almost constant for a particular species at a specific developmental stage (Cui and Benfey, 2009). These changes are usually accompanied by a thickening of plant cell walls when exposed to metal(loid) stress as observed in other nodulated legumes such as *Medicago truncatula* and *Anthyllis vulneraria*, growing under Al and As or Pb and Zn, respectively (Krzeslowska, 2011; Sujkowska-Rybkowska *et al*., 2012; 2015; Gall *et al*., 2015; Lafuente *et al*., 2015; Sujkowska-Rybkowska and Ważny, 2018). Thus, the plant cell wall constitutes an important defense barrier against the entry of toxic ions avoiding or limiting their mobilization throughout the plant (Probst *et al*., 2009; Ovečka and Takáč, 2014; Gall *et al*. 2015). However, when plants are exposed to abiotic stress that induces oxidative stress, roots could increase the number of layers in the cortex in order to reduce ROS-toxicity (Cui, 2015). In addition, it is widely known that once As is inside the cells, it is rapidly complexed by glutathione (GSH) and phytochelatins (PCs) in order to compartmentalize the metalloid into the vacuole (Zhao *et al*., 2009). Thus, in peanut plants, the increase of the number of cortex layers would imply that there is a greater number of vacuoles that can compartmentalize the metalloid in order to avoid its entry into the vascular tissue and therefore, reach aerial tissues.

Photosynthesis is a process commonly affected by As. This could be due to alterations in the chlorophyll content and biosynthesis, and the reduction of photosystem II activity, among others (Gusman *et al*., 2013; Suneja *et al*., 2014; Emamverdian *et al*., 2015; Hasanuzzaman *et al*., 2017). In plant cells, chloroplasts are the main organelle where ROS are generated. Under normal conditions, the electron flows from the reaction

centers of the excited photosystem to $NADP^+$, reducing it to NADPH. Finally, NADPH reduces CO_2 in the Calvin cycle (Shikanai, 2007). Although there are no studies that demonstrate the intrinsic mechanisms of As toxicity at the photosynthetic apparatus level until now, it is suggested that it could be related to an alteration of the redox homeostasis (Finnegan and Chen, 2012). Under several stress conditions, when CO_2 fixation is limited, the overload of the electron transport chain produces a leak of ferredoxin electrons to O_2, giving rise to the oxidative burst which may affect chlorophyll integrity (Sharma *et al*., 2012 b; Kostecka-Gugała and Latowski, 2018). Thus, some authors proposed the determination of chlorophyll content as a redox status index (Miteva, 2002; Shaibur *et al*., 2009). While As induced-chlorophyll damage is a widely reported response (Stoeva *et al*., 2003; Abbas *et al*., 2018), a study performed by Mascher *et al*. (2002), showed that no modification of chlorophyll content was observed in red clover plants, when exposed to a low As concentration. In addition, an increment in chlorophyll content was observed in *Solanum lycopersicum* plants exposed to low As^V concentrations (Miteva, 2002). A similar behavior was observed in *Allium cepa* and in *Zea maize* plants subjected to metalloid exposure (Singh Sushant and Ghosh, 2010; Mallick *et al*., 2011). On the other hand, carotenoids protect light-harvesting complexes against light over-excitation and photo-oxidative damage. It is well documented that they dissipate the excess of absorbed energy, thereby preventing or reducing the formation of ROS such as $^1O_2$ (Strzałka *et al*., 2003) even in plants growing under As exposure (Neill *et al*., 2002; Sinha *et al*., 2013). In this work, As^V induced a significant increase in the content of photosynthetic pigments in peanut plants inoculated with *Bradyrhizobium* sp. C-145. This result was in agreement with our previous findings in which peanut inoculation with *Bradyrhizobium*sp. C-145 promoted plant growth even when the metalloid translocation to the aerial part was higher, compared to peanut plants inoculated with *Bradyrhizobium* sp. SEMIA6144 (Peralta *et al*., 2019). Thus, it is possible to suggest that the increase in pigment content could be a protective defense mechanism against As, in order to maintain a vital process in plant cells, such as carbon fixation.

The over-production and/or accumulation of ROS, including $O_2^{\cdot -}$, •OH and H_2O_2 is a common response of plants exposed to As (Sharma *et al*., 2012 b; Bianucci *et al*., 2017; 2019). However, the alteration of the redox status of cells induced by metal(loid)s depends on several variables such as, the stages of plant growth and the plant species, the time of exposure, the type of substrate used and the concentration of the contaminant, among others. In this sense, Gupta *et al.* (2013) reported an increase in H_2O_2 content in *Arabidopsis thaliana*

plants grown for five days in a hydroponic system, supplemented with 50 µM As^V. In the same way, peanut plants exposed to 20 and 100 µM As^V for fifteen days, showed an increase in $O_2^{\cdot -}$ and H_2O_2 contents compared to the control condition, without significant ROS production at the lower concentration tested (6 µM As^V) (Bianucci *et al*., 2017). Our results showed that the exposition of peanut plant to 3 µM As^V, for a period of 30 days in a semi-hydroponic system, induced a significant oxidative burst. Among the possible causes of ROS generation in plants, the activity of NADPH oxidase stands out. This enzyme is a transmembrane flavoprotein complex that oxidizes cytoplasmic NADPH, translocates electrons across the plasma membrane, and reduces extracellular oxygen to $O_2^{\bullet -}$. Then, it is rapidly dismuted into H_2O_2 species (Torres and Danlg, 2005) and, it has been demonstrated that it is strongly activated by As (Hernández *et al*., 2015; Bianucci *et al*., 2017). In accordance with the above presented findings, our results revealed that the activity of NADPH oxidase was significantly enhanced by a realistic concentration of As in peanut plants, irrespective of the strain used as inoculant. Also, the radical $O_2^{\cdot -}$ was prominent in leaves and roots of peanut and, the H_2O_2 content was significantly increased too. ROS over-production and/or accumulation in peanut plants could be due to an alteration in the antioxidant system capacity, represented by an increased superoxide dismutase (SOD) activity, a decrease in catalase (CAT) or glutathione peroxidase/peroxirredoxine (GPX/PRX) activities, a depletion of GSH and proteins rich in sulfhydryl groups (SH-), or by a combination of these (Das and Roychoudhury, 2014; Choudhury *et al*., 2017; Bianucci *et al*., 2017). Thus, when the generation or accumulation of ROS exceeds the detoxification ability of cells, an oxidative stress is produced, which is manifested by the oxidation of essential biomolecules (Scandalios *et al*., 2002). Many studies propose lipid peroxidation as the most severe oxidative process in plants exposed to As (Parkhey *et al*., 2012; Shahid *et al*., 2014; Souri *et al*., 2017; Abbas *et al*., 2018). This stress can damage cell membranes, leading to high leakage of cellular electrolytes and essential components of the normal cellular metabolism (Gill and Tuteja, 2010; Clemens and Ma, 2016). One of the most studied lipid peroxidation by-product is TBARS content and it has been the main stress index used so far (Gill and Tuteja, 2010). Gupta *et al*. (2013) reported that As increased peroxidation of lipids in the leaves at the early stages of growth of *Arabidopsis* plants exposed to As, although statistically significant differences have only been observed after a long treatment. Both, lipid peroxidation and electrolyte leakage were significantly increased in leaves and roots of *Trigonella foenumgraecum* legume when exposed to 90 µM As^V (Talukdar, 2013). In a recent study, Singh *et al*. (2017)

364 revealed that *Vetiveria zizanoides* plants exposed to increasing concentrations of arsenic (0-200 µM) showed a
365 correlated increase in lipid peroxidation (TBARS). As we previously mentioned, besides lipids, ROS can
366 oxidize proteins as well and, although both inorganic arsenic species are toxic to protein components, As^{III}
367 (generated by As^{V} reduction) was described as the most harmful species (Mishra and Dubey, 2006). Thereby,
368 ROS generated in response to arsenic stress can modify the structures of the proteins demonstrated by the
369 release of carbonyl groups (Parkhey *et al*., 2014). So far, particularly in plants, it has not been determined that
370 the induction of oxidation/carbonylation of proteins is promoted by As; several researches have focused on
371 other metals: Co (Karuppanapandian and Kim, 2013), Zn (Ramakrishna and Rao, 2013), Cd (Romero-Puertas
372 *et al*., 2002; Rodríguez-Serrano *et al*., 2006; Bianucci *et al*., 2012; Pérez-Chaca *et al*., 2014; Xu *et al*., 2018)
373 and Cu (Mustafa and Komatsu, 2016; Ju *et al*., 2019).
374 Taken together, our results revealed that for both inoculation conditions, the oxidative burst in peanut plants
375 exposed to a natural concentration of As^{V}, corresponds to the oxidative damage evidenced by the increase in
376 TBARS content and carbonyl proteins. One of the most relevant findings was that the redox response was
377 strain-dependent and organ-differential. Thus, for the symbiotic pair peanut-*Bradyrhizobium* sp. SEMIA6144,
378 a higher H_2O_2 content and damage to lipid components was detected in roots, while in leaves a higher
379 superoxide anion production and damage to lipids and proteins was evidenced. On the other hand, for the
380 peanut-*Bradyrhizobium* sp.C-145 interaction, a high NADPH oxidase activity was observed in roots which
381 may be associated with lipid peroxidation and carbonylated proteins, while in leaves an increase in H_2O_2
382 content was highlighted. It is necessary to emphasize that literature has a wide background about the high
383 arsenic concentration effects on non-hyperaccumulating plants. Usually, the changes detected are as expected
384 for such concentration (Abbas *et al*., 2018).
385 Although originally the inoculation of symbiotic microorganisms had a sole and important function, to serve
386 as a nitrogen source for legumes in order to improve growth and increase yield, the uses of such
387 microorganisms as bioremediation tools constitutes an additional function that has gained special attention in
388 the last ten years (Reichman, 2007; Mandal *et al*., 2011; Gómez-Sagasti and Marino, 2015). In addition,
389 physico-chemical transformations of metal(oid)s carried out by microorganisms in the rhizosphere depend on
390 their intrinsic capacities (Bhattacharjee and Rosen 2007; Finnegan and Chen 2012; Lomax *et al*., 2012; Yang
391 and Rosen, 2016). For this reason, the selection of the best rhizobia partnership to the legume requires deep

researches, since not all rhizobia act in a same manner when inoculated to plants exposed to different As concentrations. In particular, the symbiotic interaction could not only modify the growth variables of the macrosymbiont, but also to enhance the phytostabilization processes serving as a promising biotechnological strategy to avoid metal(oid)s translocation to edible parts (Bianucci *et al*., 2018; Peralta *et al*., 2019; Bianucci *et al*., 2020). Accordingly, legume inoculation should be carefully controlled, especially in crops whose grains and their post-processing waste are used to produce food for humans or livestock (Gómez-Sagasti and Marino, 2015). The key contribution of this work is the elucidation that a low concentration of As^{V}, naturally present in groundwater of cropping areas from Argentina, negatively impacts on the redox state of peanut plants, which is a novel observation. In addition, each rhizobial strain participates in the modulation of the redox response on the symbiotic system in a specific way, conditioning the crop growth and the arsenic translocation to the edible parts of the legume, as previously observed (Peralta *et al*., 2019).

5. Concluding remarks and perspectives

In order to deepen the As toxicity effect on peanut plants, the present work is shedding light on the fact that a realistic concentration of the metalloid generates an oxidative burst by increased ROS levels and oxidative stress due to damage to essential biomolecules. The proposed model from this work highlights that the redox response of the peanut-rhizobia system is modulated by the intrinsic As tolerance of rhizobial strains. These findings, along with our previous ones, provides the necessary tools for choosing an effective formulation of bioinoculants to be used in peanut crops exposed to a naturally adverse condition at field. Studies currently underway are seeking to unravel the antioxidant response in order to elucidate whether inoculation generates a strain-specific response.

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Conflict of interest

None of the authors has any conflict of interest to declare.

7. References

Abbas, G., Murtaza, B., Bibi, I., Shahid, M., Niazi, N.K., Khan, M.I., Hussain, M., 2018. Arsenic uptake, toxicity, detoxification, and speciation in plants: physiological, biochemical, and molecular aspects. *International Journal of Environmental Research and Public Health*. 15(1), 59. DOI: https://doi.org/10.3390/ijerph15010059

Agriexchange, 2017. http://agriexchange.apeda.gov.in/ (accessed Sept 2018).

Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell & Environment*. 24(12), 1337-1344. DOI: https://doi.org/10.1046/j.1365-3040.2001.00778.x

Argentine Peanut Chamber, 2012. National and international market statistics. www.camaraargentinadelmani.com.ar (accessed July 2020).

Begum, M.C., Islam, M.S., Islam, M., Amin, R., Parvez, M.S., Kabir, A.H., 2016. Biochemical and molecular responses underlying differential arsenic tolerance in rice (*Oryza sativa* L.). *Plant Physiology and Biochemistry*. 104, 266-277. DOI: https://doi.org/10.1016/j.plaphy.2016.03.034

Bhattacharjee, H., Rosen, B.P., 2007. Arsenic metabolism in prokaryotic and eukaryotic microbes. In: Nies, D.H., Silver, S. (Eds.), Molecular Microbiology of Heavy Metals. Microbiology Monographs. Springer, Berlín, Heidelberg, (6), 371-406. DOI: https://doi.org/10.1007/7171_2006_086

Bianucci, E., Furlan, A., del Carmen Tordable, M., Hernández, L.E., Carpena-Ruiz, R.O., Castro, S., 2017. Antioxidant responses of peanut roots exposed to realistic groundwater doses of arsenate:

Identification of glutathione S-transferase as a suitable biomarker for metalloid toxicity. *Chemosphere*. 181, 551-561. DOI: https://doi.org/10.1016/j.chemosphere.2017.04.104

Bianucci, E., Furlan, A., Hernández, L.E., Castro, S., 2019. Insights into the Effect of a Natural Arsenate Dose on Growth, Nodulation and Redox Metabolism of Soybean Plants. *Pedosphere*. 29(4), 527-533.

Bianucci, E., Furlan, A., Isaia, A., Peralta, J.M., Hernandez, L.E., Castro, S., 2016. Impact of arsenic in Bradyrhizobia strains and in the symbiotic interaction with peanut plant. Supplement. 2 52th Annual Meeting Argentine Society for Biochemistry and Molecular Biology. *BIOCELL*. 40 (Suppl. S), 113.

Bianucci, E., Godoy, A., Furlan, A., Peralta, J.M., Hernández, L.E., Carpena-Ruiz, R.O., Castro, S., 2018. Arsenic toxicity in soybean alleviated by a symbiotic species of *Bradyrhizobium*. *Symbiosis*. 74(3), 167-176. DOI: https://doi.org/10.1007/s13199-017-0499-y

Bianucci, E., Peralta, J.M., Furlan, A., Hernández, L.E., Castro, S., 2020. Arsenic in Wheat, Maize, and Other Crops, in: Srivastava, S. (Eds.) Arsenic in Drinking Water and Food. Springer, Singapore, 279-306. DOI: https://doi.org/10.1007/978-981-13-8587-2_9

Blarasin, M., Cabrera, A., Matteoda, E., 2014. Aguas subterráneas de la provincia de Córdoba¸ Universidad Nacional de Río Cuarto, Argentina [Groundwater of Córdoba province, National University of Río Cuarto].

Bolan, N.S., Park, J.H., Robinson, B., Naidu, R., Huh, K.Y., 2011. Phytostabilization: a green approach to contaminant containment. In: Donald L. Sparks (Ed), Advances in agronomy. Academic Press, (112), 145-204). DOI: https://doi.org/10.1016/B978-0-12-385538-1.00004-4

Boote, K.J., 1982. Growth stages of peanut (*Arachis hypogaea* L.). *Peanut Science*. 9(1), 35-40. DOI: https://doi.org/10.3146/i0095-3679-9-1-11

Bustingorri, C., Lavado, R.S., 2014. Soybean as affected by high concentrations of arsenic and fluoride in irrigation water in controlled conditions. *Agricultural Water Management*. (144), 134-139. DOI: https://doi.org/10.1016/j.agwat.2014.06.004

Cabrera, A., Blarasin, M., Matteoda, E., Villalba, G., Gómez, G.M., 2005. Argentine Site of Animal Production. www.produccion-animal.com.ar (accessed July 2015).

Catarecha, P., Segura, M.D., Franco-Zorrilla, J.M., García-Ponce, B., Lanza, M., Solano, R., Leyva, A., 2007. A mutant of the *Arabidopsis* phosphate transporter PHT1; 1 displays enhanced arsenic accumulation. *The Plant Cell*. 19(3), 1123-1133. DOI: https://doi.org/10.1105/tpc.106.041871

Choudhury, R., Mahanta, C., Verma, S., Mukherjee, A., 2017. Arsenic distribution along different hydrogeomorphic zones in parts of the Brahmaputra River Valley, Assam (India). *Hydrogeology Journal*. 25(4), 1153-1163. DOI: https://doi.org/10.1007/s10040-017-1584-2

Cui, H., 2015. Cortex proliferation in the root is a protective mechanism against abiotic stress. *Plant Signaling & Behavior*. 10(5), e1011949. DOI: https://doi.org/10.1080/15592324.2015.1011949

Cui, H., Benfey, P.N., 2009. Cortex proliferation: simple phenotype, complex regulatory mechanisms. *Plant Signaling & Behavior*. 4(6), 551-553. DOI: https://doi.org/10.4161/psb.4.6.8731

Dary, M., Chamber-Pérez, M. A., Palomares, A. J., Pajuelo, E., 2010. "*In situ*" phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. *Journal of Hazardous Materials*. 177(1-3), 323-330. DOI: https://doi.org/10.1016/j.jhazmat.2009.12.035

Das, K., Roychoudhury, A., 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science*. 2,53. DOI: https://doi.org/10.3389/fenvs.2014.00053

Delgadillo, J., Lafuente, A., Doukkali, B., Redondo-Gómez, S., Mateos-Naranjo, E., Caviedes, M.A., Rodríguez-Llorente, I.D., 2015. Improving legume nodulation and Cu rhizostabilization using a genetically modified rhizobia. *Environmental Technology* (United Kingdom). 36(10), 1237–1245. DOI: https://doi.org/10.1080/09593330.2014.983990

Deng, F., Yu, M., Martinoia, E., Song, W.Y., 2019. Ideal cereals with lower arsenic and cadmium by accurately enhancing vacuolar sequestration capacity. *Frontiers in Genetics*. 10. DOI: https://doi.org/10.3389/fgene.2019.00322

Di Rienzo, J., Casanoves, F., Balzarina, M., Gonzalez, L., Tablada, M., Robledo, C. Infostat versión 2018. Centro de Transferencia Infostat, FCA. Universidad Nacional de Córdoba. Argentina.

499 Emamverdian, A., Ding, Y., Mokhberdoran, F., Xie, Y., 2015. Heavy metal stress and some mechanisms of
500 plant defense response. *The Scientific World Journal*. 756120. DOI:
501 https://doi.org/10.1155/2015/756120
502 Fagorzi, C., Checcucci, A., DiCenzo, G. C., Debiec-Andrzejewska, K., Dziewit, L., Pini, F., Mengoni, A.,
503 2018. Harnessing rhizobia to improve heavy-metal phytoremediation by legumes. *Genes*. 9(11), 542.
504 FAOSTAT, 2016. http://faostat.fao.org/site/339/default.aspx (accessed 2 May 2019).
505 Fink, B., Laude, K., McCann, L., Doughan, A., Harrison, D.G., Dikalov, S., 2004. Detection of intracellular
506 superoxide formation in endothelial cells and intact tissues using dihydroethidium and an HPLC-
507 based assay. *American Journal of Physiology-Cell Physiology*. 287(4), C895-C902. DOI:
508 https://doi.org/10.1152/ajpcell.00028.2004
509 Finnegan, P., Chen, W., 2012. Arsenic toxicity: the effects on plant metabolism. *Frontiers in Physiology*.
510 3:182. DOI: https://doi.org/10.3389/fphys.2012.00182
511 Foyer, C.H., Shigeoka, S., 2011. Understanding oxidative stress and antioxidant functions to enhance
512 photosynthesis. *Plant Physiology*. 155(1): 93-100. DOI: https://doi.org/10.1104/pp.110.166181
513 Frahry, G., Schopfer, P., 2001. NADH-stimulated, cyanide-resistant superoxide production in maize
514 coleoptiles analyzed with a tetrazolium-based assay. *Planta*. 212(2), 175-183. DOI:
515 https://doi.org/10.1007/s004250000376
516 Francisca, F.M., Celollada-Verdaguer, M.P., Carro-Pèrez, M.E., 2006. Presented in part at Conference VIII
517 Congreso Latinoamericano de hidrología subterránea. Distribución espacial del arsénico en las aguas
518 subterráneas de la provincia de Córdoba, Argentina. Asunción del Paraguay.
519 Gall, H., Philippe, F., Domon, J. M., Gillet, F., Pelloux, J., Rayon, C., 2015. Cell wall metabolism in response
520 to abiotic stress. *Plants*. 4(1), 112-166. DOI: https://doi.org/10.3390/plants4010112
521 Ghosh, S., Shaw, A.K., Azahar, I., Adhikari, S., Jana, S., Roy, S., Hossain, Z., 2016. Arsenate (AsV) stress
522 response in maize (*Zea mays* L.). *Environmental and Experimental Botany*.130, 53-67. DOI:
523 https://doi.org/10.1016/j.envexpbot.2016.05.003
524 Gill, S. S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in
525 crop plants. *Plant Physiology and Biochemistry*. 48(12), 909-930. DOI:
526 https://doi.org/10.1016/j.plaphy.2010.08.016

527 Gómez-Sagasti, M. T., Marino, D., 2015. PGPRs and nitrogen-fixing legumes: a perfect team for efficient Cd
528 phytoremediation? *Frontiers in Plant Science*. 6, 81. DOI: https://doi.org/10.3389/fpls.2015.00081
529 Gupta, D.K., Inouhe, M., Rodríguez-Serrano, M., Romero-Puertas, M.C., Sandalio, L.M., 2013. Oxidative
530 stress and arsenic toxicity: role of NADPH oxidases. *Chemosphere*. 90(6): 1987-1996. DOI:
531 https://doi.org/10.1016/j.chemosphere.2012.10.066
532 Gusman, G.S., Oliveira, J.A., Farnese, F.S., Cambraia, J., 2013. Arsenate and arsenite: the toxic effects on
533 photosynthesis and growth of lettuce plants. *Acta Physiologiae Plantarum*. 35(4): 1201-1209. DOI:
534 https://doi.org/10.1007/s11738-012-1159-8
535 Hasanuzzaman, M., Nahar, K., Rahman, A., Al Mahmud, J., Hossain, S., Alam, K., Oku, H., Fujita, M., 2017.
536 Actions of Biological Trace Elements in Plant Abiotic Stress Tolerance. In: Naeem M., Ansari A.,
537 Gill S. (Eds.), Essential Plant Nutrients. Springer, Berlin, Germany, 213–274. DOI:
538 https://doi.org/10.1007/978-3-319-58841-4_10
539 Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of
540 fatty acid peroxidation. *Archives of Biochemistry and Biophysics*. 125(1): 189-198. DOI:
541 https://doi.org/10.1016/0003-9861(68)90654-1
542 Hernández, L.E., Sobrino-Plata, J., Montero-Palmero, M.B., Carrasco-Gil, S., Flores-Cáceres, M.L., Ortega-
543 Villasante, C., Escobar, C., 2015. Contribution of glutathione to the control of cellular redox
544 homeostasis under toxic metal and metalloid stress. *Journal of Experimental Botany*. 66(10), 2901-
545 2911. DOI: https://doi.org/10.1093/jxb/erv063
546 Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. Circular.
547 California agricultural experiment station. 347 (2nd edit).
548 Ibañez, F., Wall, L., Fabra, A., 2016. Starting points in plant-bacteria nitrogen-fixing symbioses: intercellular
549 invasion of the roots. *Journal of Experimental Botany*. 68(8), 1905-1918. DOI:
550 https://doi.org/10.1093/jxb/erw387
551 Johansen, D.A., 1940. Plant microtechique, McGraw-Hill Book Company, Inc., London.
552 Ju, W., Liu, L., Fang, L., Cui, Y., Duan, C., Wu, H., 2019. Impact of co-inoculation with plant-growth-
553 promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in copper

554 contaminated soil. *Ecotoxicology and Environmental Safety*. 167, 218-226. DOI:
555 https://doi.org/10.1016/j.ecoenv.2018.10.016
556 Karuppanapandian, T., Kim, W., 2013. Cobalt-induced oxidative stress causes growth inhibition associated
557 with enhanced lipid peroxidation and activates antioxidant responses in Indian mustard (*Brassica*
558 *juncea* L.) leaves. *Acta Physiologiae Plantarum*. 35(8), 2429-2443. DOI:
559 https://doi.org/10.1007/s11738-013-1277-y
560 Kidd, P. S., Alvarez-Lopez, V., Becerra-Castro, C., Cabello-Conejo, M., Prieto-Fernandez, A., 2017. Potential
561 role of plant-associated bacteria in plant metal uptake and implications in phytotechnologies. In: Ann
562 Cuypers, Jaco Vangronsveld (Eds.), Advances in botanical research. Academic Press, 83, 87-126.
563 DOI: https://doi.org/10.1016/bs.abr.2016.12.004
564 Kong, Z., Glick, B.R., 2017. The role of plant growth-promoting bacteria in metal phytoremediation. In:
565 Robert K. Poole (Ed), Advances in microbial physiology. Academic Press, 71, 97-132. DOI:
566 https://doi.org/10.1016/bs.ampbs.2017.04.001
567 Kostecka-Gugała, A., Latowski, D., 2018. Arsenic-induced oxidative stress in plants. In: Hasanuzzaman, M.,
568 Nahar, K., Fujita, M. (Eds.), Mechanisms of Arsenic Toxicity and Tolerance in Plants. Springer,
569 Singapore, 79-104. DOI: https://doi.org/10.1007/978-981-13-1292-2
570 Krzesłowska, M., 2011. The cell wall in plant cell response to trace metals: polysaccharide remodeling and its
571 role in defense strategy. *Acta Physiologiae Plantarum*. 33(1), 35-51. DOI:
572 https://doi.org/10.1007/s11738-010-0581-z
573 Lafuente, A., Pérez-Palacios, P., Doukkali, B., Molina-Sánchez, M.D., Jiménez-Zurdo, J.I., Caviedes, M.A.,
574 Pajuelo, E., 2015. Unraveling the effect of arsenic on the model *Medicago–Ensifer* interaction: a
575 transcriptomic meta-analysis. *New Phytologist*. 205(1), 255-272. DOI:
576 https://doi.org/10.1111/nph.13009
577 Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Stadtman, E.R., 1990.
578 Determination of carbonyl content in oxidatively modified proteins, In: Methods in enzymology. 186,
579 464-478. DOI: https://doi.org/10.1016/0076-6879(90)86141-H

Lomax, C., Liu, W.J., Wu, L., Xue, K., Xiong, J., Zhou, J., Zhao, F.J., 2012. Methylated arsenic species in plants originate from soil microorganisms. *New Phytologist*. 193(3), 665-672. DOI: https://doi.org/10.1111/j.1469-8137.2011.03956.x

Mac Kinney, G., 1938. Some absorption spectra of leaf extracts. *Plant Physiology*. 13(1), 123. DOI: https://doi.org/10.1104/pp.13.1.123

Mandal, S.M., Gouri, S.S., De, D., Das, B.K., Mondal, K.C., Pati, B.R., 2011. Effect of arsenic on nodulation and nitrogen fixation of blackgram (*Vigna mungo*). *Indian Journal of Microbiology*. 51(1):44-47. DOI: https://doi.org/10.1007/s12088-011-0080-y

Mishra, S., Dubey, R.S., 2006. Inhibition of ribonuclease and protease activities in arsenic exposed rice seedlings: role of proline as enzyme protectant. *Journal of Plant Physiology*. 163(9), 927-936. DOI: https://doi.org/10.1016/j.jplph.2005.08.003

Miteva, E., 2002. Accumulation and effect of arsenic on tomatoes. *Communications in Soil Science and Plant Analysis*. 33(11-12), 1917-1926. DOI: https://doi.org/10.1081/CSS-120004832

Mittler, R., 2017. ROS are good. *Trends in Plant Sci*ence. 22(1), 11-19. DOI: https://10.1016/j.tplants.2016.08.002

Mittler, R., Vanderauwera, S., Suzuki, N., Miller, G.A.D., Tognetti, V.B., Vandepoele, K., Van Breusegem, F., 2011. ROS signaling: the new wave? *Trends in Plant Science*. 16(6), 300-309. DOI: https://doi.org/10.1016/j.tplants.2011.03.007

Mustafa, G., Komatsu, S., 2016. Toxicity of heavy metals and metal-containing nanoparticles on plants. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*. 1864(8), 932-944. DOI: https://doi.org/10.1016/j.bbapap.2016.02.020

Neill, S.J., Desikan, R., Clarke, A., Hurst, R.D., Hancock, J.T., 2002. Hydrogen peroxide and nitric oxide as signalling molecules in plants. *Journal of Experimental Botany*. 53(372), 1237-1247. DOI: https://doi.org/10.1093/jexbot/53.372.1237

Nordstrom, D.K., 2002. Worldwide occurrences of arsenic in ground water. 2143-2145. DOI: https://doi.org/10.1126/science.1072375

606 Orozco-Cárdenas, M., Ryan, C.A., 1999. Hydrogen peroxide is generated systemically in plant leaves by
607 wounding and system in via the octadecanoid pathway. *Proceedings of the National Academy of*
608 *Sciences*. 96(11), 6553-6557. DOI: https://doi.org/10.1073/pnas.96.11.6553
609 Ovečka, M., Takáč, T., 2014. Managing heavy metal toxicity stress in plants: biological and biotechnological
610 tools. *Biotechnology Advances*. 32(1), 73-86. DOI: https://doi.org/10.1016/j.biotechadv.2013.11.011
611 Parkhey, S., Naithani, S.C., Keshavkant, S., 2012. ROS production and lipid catabolism in desiccating *Shorea*
612 *robusta* seeds during aging. *Plant Physiology and Biochemistry*. 57, 261-267. DOI:
613 https://doi.org/10.1016/j.plaphy.2012.06.008
614 Parkhey, S., Naithani, S.C., Keshavkant, S., 2014. Protein metabolism during natural ageing in desiccating
615 recalcitrant seeds of *Shorea robusta*. *Acta Physiologiae Plantarum*. 36(7), 1649-1659. DOI:
616 https://doi.org/10.1007/s11738-014-1540-x
617 Pasternak, T., Rudas, V., Potters, G., Jansen, M.A., 2005. Morphogenic effects of abiotic stress: reorientation
618 of growth in *Arabidopsis thaliana* seedlings. *Environmental and Experimental Botany*. 53(3), 299-
619 314. DOI: https://doi.org/10.1016/j.envexpbot.2004.04.009
620 Pedelini, R., 2014. Maní. Guía práctica para su cultivo. Boletín de divulgación Técnica, INTA eds, (3): 2-19.
621 Peralta, J.M., Travaglia, C.N., Gil, R.A., Furlan, A., Castro, S., Bianucci, E.C., 2019. An effective
622 rhizoinoculation restraints arsenic translocation in peanut and maize plants exposed to a realistic
623 groundwater metalloid dose, in: Zhu, Y., Guo, H., Bhattacharya, P., Ahmad, A., Bundschuh, J.,
624 Naidu, R. (Eds.), Environmental Arsenic in a Changing World: Proceedings of the 7th International
625 Congress and Exhibition on Arsenic in the Environment (AS 2018).CRC Press, Beijing, PR China, p.
626 283. DOI: https://doi.org/10.1201/9781351046633-112
627 Pérez-Chaca, M.V., Rodríguez-Serrano, M., Molina, A.S., Pedranzani, H.E., Zirulnik, F., Sandalio, L.M.,
628 Romero-Puertas, M.C., 2014. Cadmium induces two waves of reactive oxygen species in *Glycine max*
629 (L.) roots. *Plant, Cell & Environment*. 37(7), 1672-1687. DOI: https://doi.org/10.1111/pce.12280
630 Pickering, I.J., Prince, R.C., George, M.J., Smith, R.D., George, G.N., Salt, D.E., 2000. Reduction and
631 coordination of arsenic in Indian mustard. *Plant Physiology*. 122(4), 1171-1178. DOI:
632 https://doi.org/10.1104/pp.122.4.1171

633 Probst, A., Liu, H., Fanjul, M., Liao, B., Hollande, E., 2009. Response of *Vicia faba* L. to metal toxicity on
634 mine tailing substrate: geochemical and morphological changes in leaf and root. *Environmental and*
635 *Experimental Botany*. 66(2), 297-308. DOI: https://doi.org/10.1016/j.envexpbot.2009.02.003
636 Ramakrishna, B., Rao, S.S.R., 2013. Preliminary studies on the involvement of glutathione metabolism and
637 redox status against zinc toxicity in radish seedlings by 28-Homobrassinolide. *Environmental and*
638 *Experimental Botany*. 96, 52-58. DOI: https://doi.org/10.1016/j.envexpbot.2013.08.003
639 Reichman, S.M., 2007. The potential use of the legume–rhizobium symbiosis for the remediation of arsenic
640 contaminated sites. *Soil Biology and Biochemistry*. 39(10), 2587-2593. DOI:
641 https://doi.org/10.1016/j.soilbio.2007.04.030
642 Rodríguez-Serrano, M., Romero-Puertas, M.C., Zabalza, A., Corpas, F.J., Gomez, M., Del Rio, L.A.,
643 Sandalio, L.M., 2006. Cadmium effect on oxidative metabolism of pea (*Pisum sativum* L.) roots.
644 Imaging of reactive oxygen species and nitric oxide accumulation in vivo. *Plant, Cell & Environment*.
645 29(8), 1532-1544. DOI: https://doi.org/10.1111/j.1365-3040.2006.01531.x
646 Romero-Puertas, M.C., Palma, J.M., Gómez, M., Del Rio, L.A., Sandalio, L.M., 2002. Cadmium causes the
647 oxidative modification of proteins in pea plants. *Plant, Cell & Environment*. 25(5), 677-686. DOI:
648 https://doi.org/10.1046/j.1365-3040.2002.00850.x
649 Sagi, M., Fluhr, R., 2001. Superoxide production by plant homologues of the gp91phox NADPH oxidase.
650 Modulation of activity by calcium and by tobacco mosaic virus infection. *Plant Physiology*. 126(3),
651 1281-1290. DOI: https://doi.org/10.1104/pp.126.3.1281
652 Sánchez-Bermejo, E., Castrillo, G., Del Llano, B., Navarro, C., Zarco-Fernández, S., Martínez-Herrera, D.J.,
653 Alonso-Blanco, C., 2014. Natural variation in arsenate tolerance identifies an arsenate reductase in
654 *Arabidopsis thaliana*. *Nature Communications*. 5, 4617. DOI: https://doi.org/10.1038/ncomms5617
655 Sandalio, L.M., Rodríguez-Serrano, M., Romero-Puertas, M.C., Luis, A., 2008. Imaging of reactive oxygen
656 species and nitric oxide in vivo in plant tissues. *Methods in Enzymology*. 440, 397-409. DOI:
657 https://doi.org/10.1016/S0076-6879(07)00825-7
658 Scandalios, J.G., 2002. The rise of ROS. *Trends in Biochemical Sciences*. 27(9), 483-486. DOI:
659 https://doi.org/10.1016/s0968-0004(02)02170-9

Schikora, A., Schmidt, W., 2001. Acclimative changes in root epidermal cell fate in response to Fe and P deficiency: a specific role for auxin?. *Protoplasma*. 218(1-2), 67-75. DOI: https://doi.org/10.1007/BF01288362

Shahid, F., Rizwan, S., Khan, M.W., Khan, S.A., Naqshbandi, A., Yusufi, A.N.K., 2014. Studies on the effect of sodium arsenate on the enzymes of carbohydrate metabolism, brush border membrane, and oxidative stress in the rat kidney. *Environmental Toxicology and Pharmacology*. 37(2), 592-599. DOI: https://doi.org/10.1016/j.etap.2014.01.012

Shahid, M., Dumat, C., Khalid, S., Schreck, E., Xiong, T., Niazi, N.K., 2017. Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake. *Journal of Hazardous Materials*. 325, 36-58. DOI: https://doi.org/10.1016/j.jhazmat.2016.11.063

Shaibur, M.R., Kitajima, N., Huq, S.I., Kawai, S., 2009. Arsenic–iron interaction: Effect of additional iron on arsenic-induced chlorosis in barley grown in water culture. *Soil Science and Plant Nutrition*. 55(6), 739-746. DOI: https://doi.org/10.1111/j.1747-0765.2009.00414.x

Sharma, I., 2012 a. Arsenic induced oxidative stress in plants. *Biologia*. 67(3), 447-453. DOI: https://doi.org/10.2478/s11756-012-0024-y

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M., 2012 b. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*. DOI: https://doi.org/10.1155/2012/217037

Shikanai, T., 2007. Cyclic electron transport around photosystem, in: Genetic Approaches. *Annual Review of Plant Biology*. 58, 199-217. DOI: https://doi.org/10.1146/annurev.arplant.58.091406.110525

Singh, H.P., Batish, D.R., Kohli, R.K., Arora, K., 2007. Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation. *Plant Growth Regulation*. 53(1), 65-73. DOI: https://doi.org/10.1007/s10725-007-9205-z

Singh, S., Sounderajan, S., Kumar, K., Fulzele, D.P., 2017. Investigation of arsenic accumulation and biochemical response of in vitro developed *Vetiveria zizanoides* plants. *Ecotoxicology and Environmental Safety*. 145, 50-56. DOI: https://doi.org/10.1016/j.ecoenv.2017.07.013

686 Sinha, K., Das, J., Pal, P.B., Sil, P.C., 2013. Oxidative stress: the mitochondria-dependent and mitochondria-
687 independent pathways of apoptosis. *Archives of Toxicology*. 87(7), 1157-1180. DOI:
688 https://doi.org/10.1007/s00204-013-1034-4
689 Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic in
690 natural waters. *Applied Geochemistry*. 17, 517-568. DOI: https://doi.org/10.1016/S0883-
691 2927(02)00018-5
692 Sobrino-Plata, J., Ortega-Villasante, C., Flores-Cáceres, M.L., Escobar, C., Del Campo, F.F., Hernández, L.E.,
693 2009. Differential alterations of antioxidant defenses as bioindicators of mercury and cadmium
694 toxicity in alfalfa. *Chemosphere*. 77(7), 946-954. DOI:
695 https://doi.org/10.1016/j.chemosphere.2009.08.007
696 Somasegaran, P., Hoben, H.J. 1994. Quantifying the growth of rhizobia, in: Handbook for Rhizobia. Springer,
697 New York. 47-57. DOI: https://doi.org/10.1007/978-1-4613-8375-8_5
698 Souri, Z., Karimi, N., Sandalio, L.M., 2017. Arsenic hyperaccumulation strategies: an overview. *Frontiers in*
699 *Cell and Developmental Biology*. 5, 67. DOI: https://doi.org/10.3389/fcell.2017.00067
700 Strzałka, K., Kostecka-Gugała, A., Latowski, D., 2003. Carotenoids and environmental stress in plants:
701 significance of carotenoid-mediated modulation of membrane physical properties. *Russian Journal of*
702 *Plant Physiology*. 50(2), 168-173. DOI: https://doi.org/10.1023/A:1022960828050
703 Sujkowska-Rybkowska, M., Borucki, W., 2015. Pectins esterification in the apoplast of aluminum-treated pea
704 root nodules. *Journal of Plant Physiology*. 184, 1-7. DOI: https://doi.org/10.1016/j.jplph.2015.05.011
705 Sujkowska-Rybkowska, M., Ważny, R., 2018. Metal resistant rhizobia and ultrastructure of Anthyllis
706 vulneraria nodules from zinc and lead contaminated tailing in Poland. *International Journal of*
707 *Phytoremediation*. 20(7), 709-720. DOI: https://doi.org/10.1080/15226514.2017.1413336
708 Sujkowska-Rybkowska, M., Borucki, W., Znojek, E., 2012. Structural changes in *Medicago truncatula* root
709 nodules caused by short-term aluminum stress. *Symbiosis*. 58(1-3), 161-170. DOI:
710 https://doi.org/10.1007/s13199-012-0211-1
711 Suneja, Y., 2014. Physio-Biochemical Responses and Allelic Diversity for Water Deficit Tolerance Related
712 Traits in *Aegilops tauschii* and *Triticum dicoccoides*. Ph.D. Thesis, Punjab Agricultural University,
713 Ludhiana, India.

714 Talukdar, D., 2013. Arsenic-induced changes in growth and antioxidant metabolism of fenugreek. *Russian*
715 *Journal of Plant Physiology*. 60(5), 652-660. DOI: https://doi.org/10.1134/S1021443713050130
716 Tarpey, M.M., Wink, D.A., Grisham, M.B., 2004. Methods for detection of reactive metabolites of oxygen
717 and nitrogen: in vitro and in vivo considerations. *American Journal of Physiology-Regulatory*,
718 *Integrative and Comparative Physiology*. 286(3), R431-R444.DOI:
719 https://doi.org/10.1152/ajpregu.00361.2003
720 Torres, M.A., Dangl, J.L., 2005. Functions of the respiratory burst oxidase in biotic interactions, abiotic stress
721 and development. *Current Opinion in Plant Biology*. 8(4), 397-403. DOI:
722 https://doi.org/10.1016/j.pbi.2005.05.014
723 Travaglia, C., Balboa, G., Espósito, G., Reinoso, H., 2012. ABA action on the production and redistribution of
724 field-grown maize carbohydrates in semiarid regions. *Plant Growth Regulation*. 67(1), 27-34. DOI:
725 https://doi.org/10.1007/s10725-012-9657-7
726 Tripathi, R.D., Srivastava, S., Mishra, S., Singh, N., Tuli, R., Gupta, D.K., Maathuis, F.J., 2007. Arsenic
727 hazards: strategies for tolerance and remediation by plants. *Trends in Biotechnology*. 25(4), 158-165.
728 DOI: https://doi.org/10.1016/j.tibtech.2007.02.003
729 Upadhyay, M.K., Yadav, P., Shukla, A., Srivastava, S., 2018. Utilizing the potential of microorganisms for
730 managing arsenic contamination: A feasible and sustainable approach. *Frontiers in Environmental*
731 *Science*. 6, 24. DOI: https://doi.org/10.3389/fenvs.2018.00024
732 Verbruggen, N., Hermans, C., Schat, H., 2009. Mechanisms to cope with arsenic or cadmium excess in plants.
733 *Current Opinion in Plant Biology*. 12(3), 364-372. DOI: https://doi.org/10.1016/j.pbi.2009.05.001
734 Vincent, J.M., 1970. A manual for the practical study of the root-nodule bacteria. A manual for the practical
735 study of the root-nodule bacteria. DOI: https://doi.org/10.1002/jobm.19720120524
736 Wang, C., Na, G., Bermejo, E.S., Chen, Y., Banks, J.A., Salt, D.E., Zhao, F.J., 2018. Dissecting the
737 components controlling root-to-shoot arsenic translocation in *Arabidopsis thaliana*. *New Phytologist*.
738 217(1), 206-218. DOI: https://doi.org/10.1111/nph.14761
739 Xu, Q., Pan, W., Zhang, R., Lu, Q., Xue, W., Wu, C., Du, S., 2018. Inoculation with *Bacillus subtilis* and
740 *Azospirillum brasilense* produces abscisic acid that reduces Irt1-mediated cadmium uptake of roots.

Journal of Agricultural and Food Chemistry. 66(20), 5229-5236. DOI: http://10.1021/acs.jafc.8b00598

Yang, H.C., Rosen, B.P., 2016. New mechanisms of bacterial arsenic resistance. *Biomedical Journal*. 39(1), 5-13. DOI: https://doi.org/10.1016/j.bj.2015.08.003

Zhao, F.J., Ma, J.F., Meharg, A.A., McGrath, S.P., 2009. Arsenic uptake and metabolism in plants. *New Phytologist*. 181(4), 777-794. DOI: https://doi.org/0.1111/j.1469-8137.2008.02716.x

Figure Legends

Figure 1. Cross section area of principal peanut root in control treatment (A and B) and exposed to 3 µM As^V (C and D), inoculated with *Bradyrhizobium* sp. SEMIA6144 (A, C) or *Bradyrhizobium* sp. C-145 (B, D). c: cortex; cc: central cylinder; en: endodermis; ep: epidermis. Magnification: 10X.

Figure 2. Effect of arsenate on NADPH oxidase activity in leaves (a) and roots (b) of peanut plants. Data represent the mean ± SE (n = 7). Different letters indicate significant differences between rhizobial strains for the same As^V dose. Different numbers indicate significant differences between As^V doses for each inoculated strain ($P < 0.05$) according to Duncan´s test.

Figure 3. Detection of hydrogen peroxide (H_2O_2) in peanut plants. (I) Representative images of H_2O_2-dependent fluorescence DCF-DA in peanut roots devoid of As^V (A and C) or exposed to 3 µM As^V (B and D). As negative control (E) the roots were incubated in 1 mM ascorbate, a H_2O_2 scavenger. Magnification: 5X. (II) Distribution of H_2O_2 in peanut leaf blade from plants devoid of As^V (A and C) and treated with 3 µM As^V (B and D). The arrows indicate the dark deposits resulting from the reaction of H_2O_2 with DAB. H_2O_2 content in peanut leaves (III) and roots (IV). Data represent the mean ± SE (n = 10). Different letters indicate significant differences between rhizobial strains for the same As^V dose. Different numbers indicate significant differences between As^V doses for each inoculated strain ($P < 0.05$) according to Duncan´s test.

Figure 4. Superoxide radical ($O_2^{\bullet -}$) production in peanut plants. (I) Histochemical detection of $O_2^{\bullet -}$ by fluorescence in the main root of peanut plants. Representative fluorescence images of $O_2^{\bullet -}$ DHE dependent staining are shown in plants devoid of As^V (A, C) or in the presence of 3 µM As^V (B, D). As a negative control (E) the roots were incubated with TMP 1 mM ($O_2^{\bullet -}$ scavenger). Magnification: 5X. (II) Distribution of $O_2^{\bullet -}$ in peanut leaf blade from plants devoid of As^V (A and C) and treated with 3 µM As^V (B and D). The arrows indicate reduced formazan blue deposits caused by the reduction of NBT with $O_2^{\bullet -}$.

Figure 5. Lipid peroxidation in leaves (A) and roots (B) of peanut plants. Data represent the mean ± SE (n = 10). Different letters indicate significant differences between rhizobial strains for the same As^V dose. Different numbers indicate significant differences between As^V doses for each inoculated strain ($P < 0.05$) according to Duncan´s test.

Figure 6. Carbonyl groups content in leaves (A) and roots (B) of peanut plants. Data represent the mean ± SE (n = 10). Different letters indicate significant differences between rhizobial strains for the same As^V dose. Different numbers indicate significant differences between As^V doses for each inoculated strain ($P < 0.05$) according to Duncan´s test.

Supplementary data

Figure S1. Schematic representation of Leonard Jar system adapted from Trung and Yoshida (1983). The superior cup (b) contained the germinated seeds (a) and the sterile support (sand: perlite, 2:1). The cup had two holes in the base through which two strips of filter paper (d) was passed. The second cup (c) contained the Hoagland´s nutrient solution devoid of As^V (control) or supplemented with 3 µM As^V.

Figure S2. Quantification of H_2O_2 fluorescence intensity (F. Int.) from images in arbitrary units (a.u.). Different letters indicate significant differences between rhizobial strains for the same As^V dose. Different numbers indicate significant differences between As^V doses for each inoculated strain ($P < 0.05$) according to Duncan´s test.

Graphical Abstract. A proposed model to explain the impact of a realistic arsenate concentration on the peanut-*Bradyrhizobium* sp. (strains SEMIA6144 or C-145) symbiotic interaction. Plant maintains or increase their photosynthetic pigment content as a possible strategy to sustain carbon fixation. Additionally, the main root increases its cross section area as a potential first restriction mechanism against metalloid entry. In leaves and roots, NADPH oxidase activity is increased along with the accumulation of superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2). Consequently, the oxidative burst can be associated with lipid and protein

damages, as revealed by the increase in TBARS and protein carbonyls content, respectively. The extent of oxidative stress is modulated by the tolerance to As of the rhizobial strain.

Table 1. Effect of arsenate on cross section area of main root of peanut plants.

	Cross section area (mm^2)			
	Total		Central cylinder	
Bradyrhizobium sp. strains	Control	3 µM As^V	Control	3 µM As^V
SEMIA6144	232.42 ± 0.10^{A1}	627.47 ± 0.15^{A2}	$28.25 \pm 0,10^{A1}$	47.24 ± 0.08^{B2}
C-145	205.55 ± 0.14^{B1}	625.25 ± 0.09^{B2}	15.25 ± 0.087^{B1}	50.36 ± 0.13^{A2}

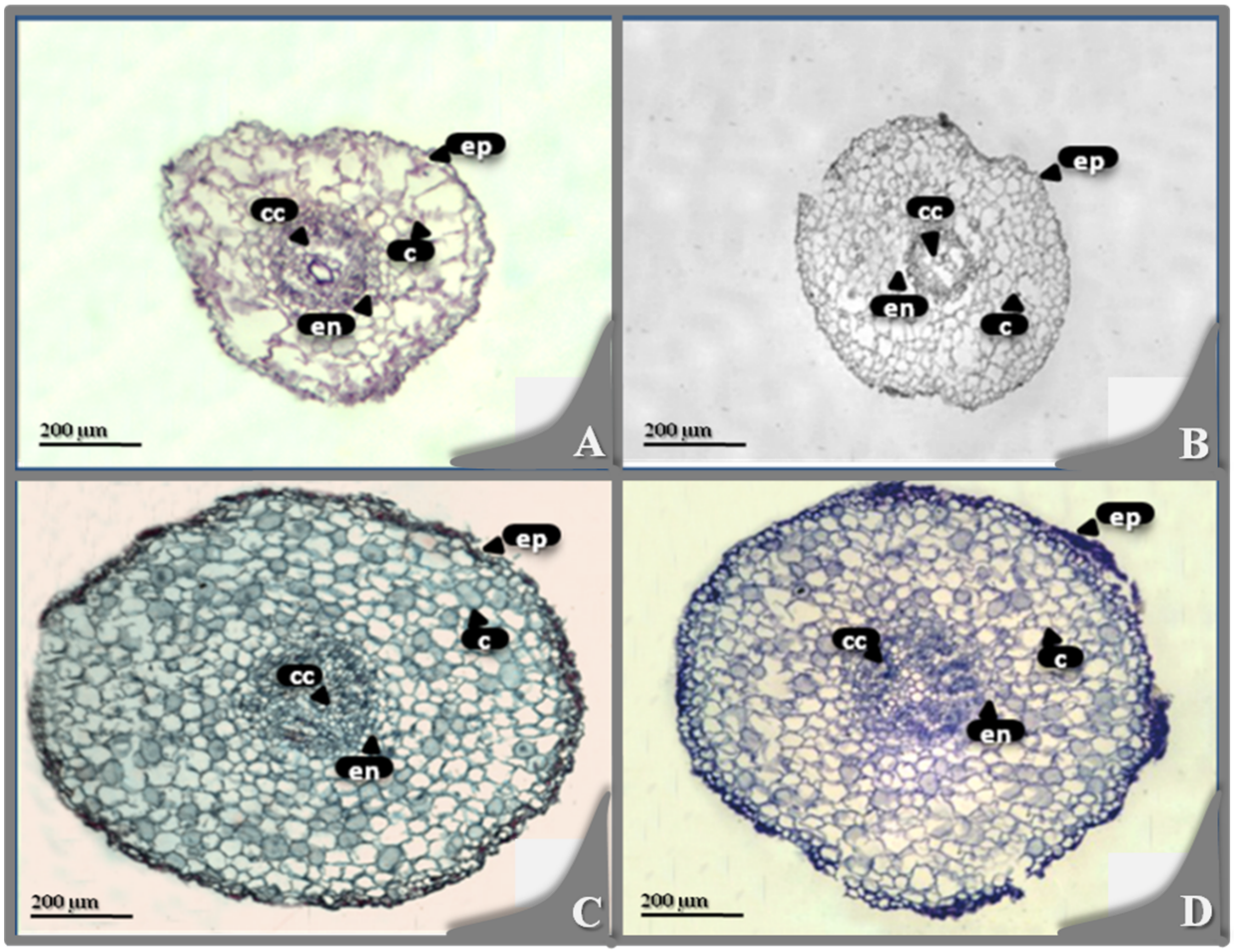
Data represent the mean ± SE (n = 10). Different letters on each column indicate significant differences between rhizobial strains for the same As^V dose. Different numbers in each row indicate significant differences between As^V doses for each inoculated strain according to the Duncan`s test ($P < 0.05$).

Table 2. Photosynthetic pigments content in peanut plants exposed to As^V.

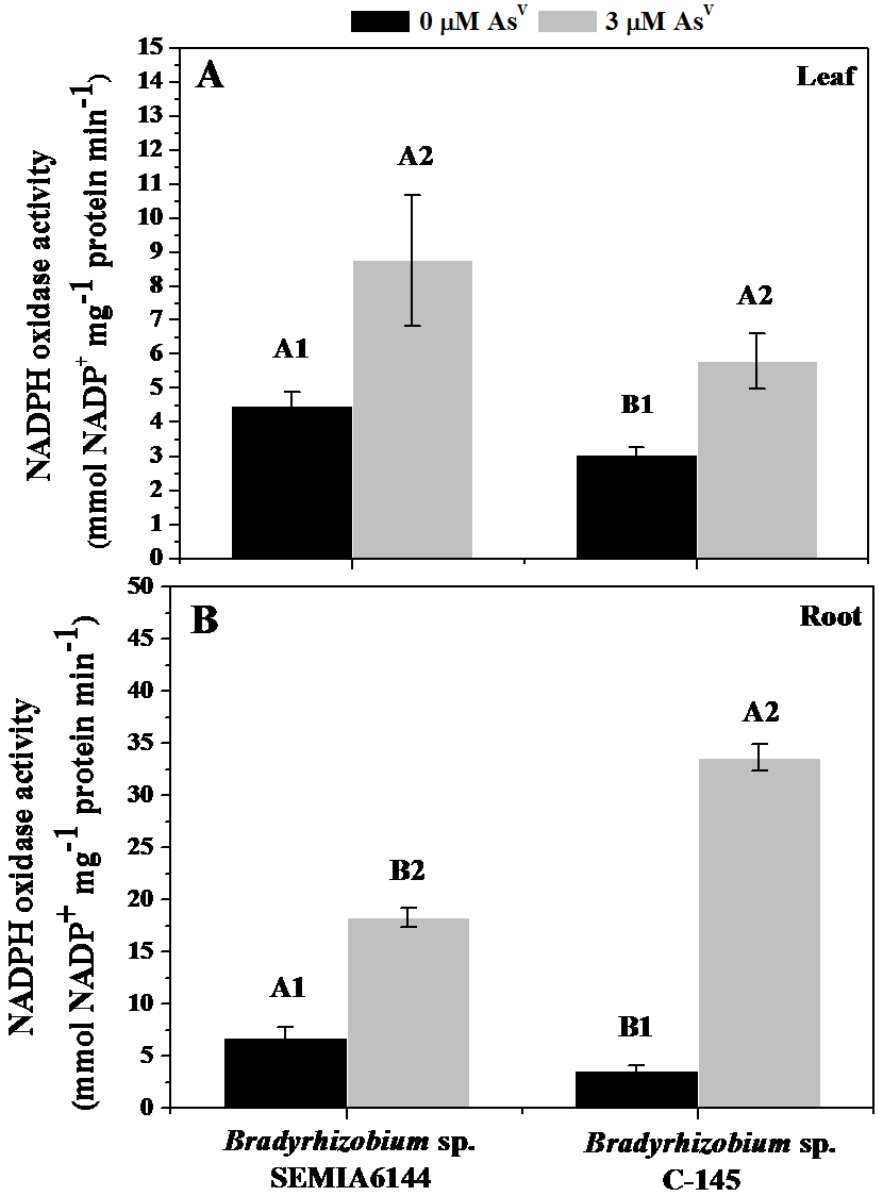
	Photosynthetic pigments content (mg g^{-1} dry weight)							
Bradyrhizobium	Total chlorophyll		Chlorophyll *a*		Chlorophyll *b*		Carotenoids	
sp. strains	Control	3 µM As^V	Control	3 µM As^V	Control	3 µM As^V	Control	3 µM As^V
SEMIA6144	$1.99 \pm 0{,}22^{A1}$	2.42 ± 0.32^{A1}	1.31 ± 0.16^{A1}	1.64 ± 0.22^{A1}	0.60 ± 0.03^{A1}	0.78 ± 0.32^{A1}	$2.6E^{-3} \pm 2.6E^{-4A1}$	$3.3E^{-3} \pm 4.5E^{-4A1}$
C-145	2.24 ± 0.28^{A1}	3.53 ± 0.26^{B2}	1.48 ± 0.19^{A1}	2.19 ± 0.12^{A2}	0.77 ± 0.09^{A1}	1.22 ± 0.12^{B2}	$2.8E^{-3} \pm 3.4E^{-4A1}$	$4.0E^{-3} \pm 2.2E^{-4B2}$

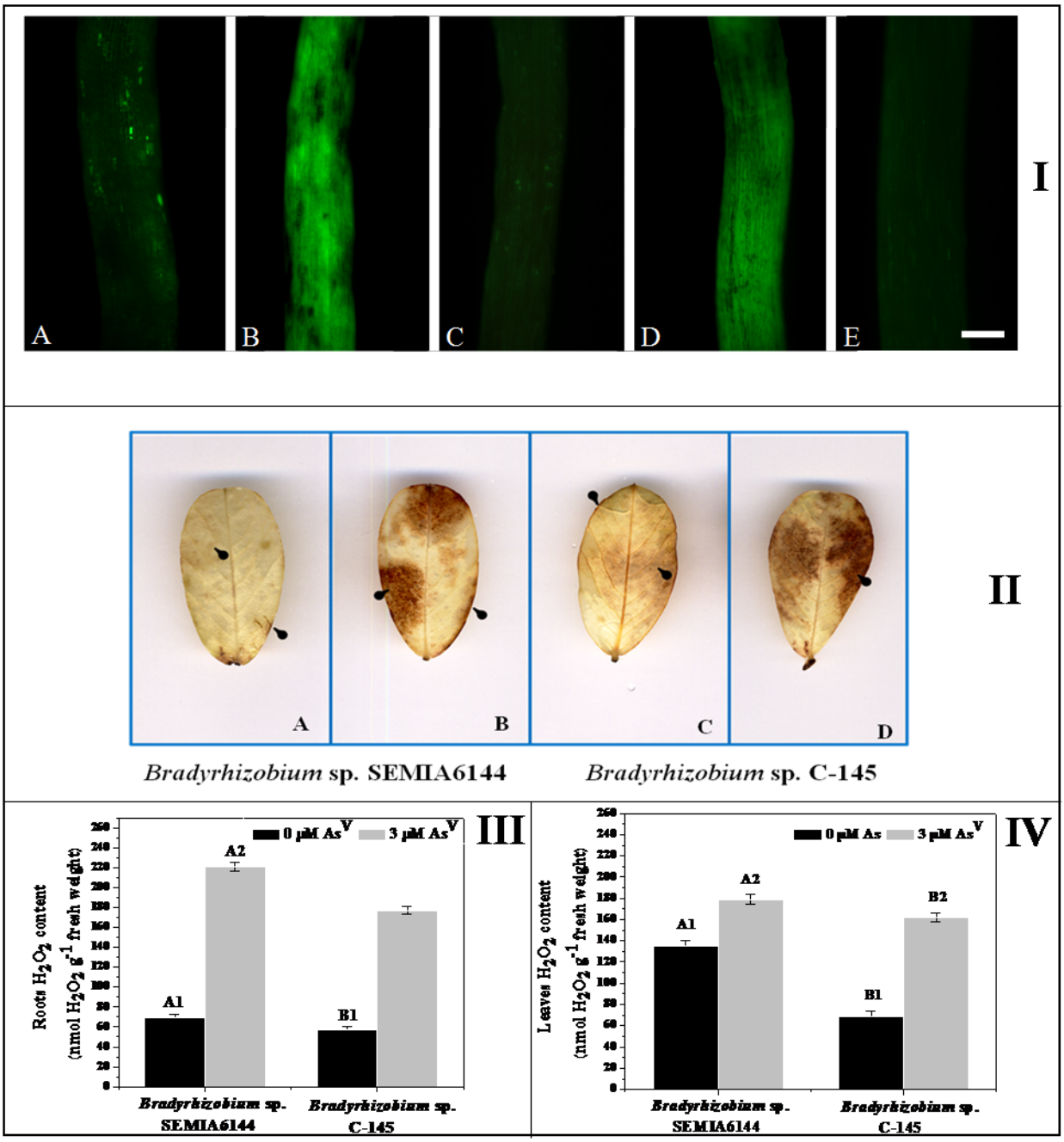
Data represent the mean ± SE (n = 10). Different letters on each column indicate significant differences between rhizobial strains for the same As^V dose. Different numbers in each row indicate significant differences between As^V doses for each inoculated strain according to the Duncan`s test ($P < 0.05$).

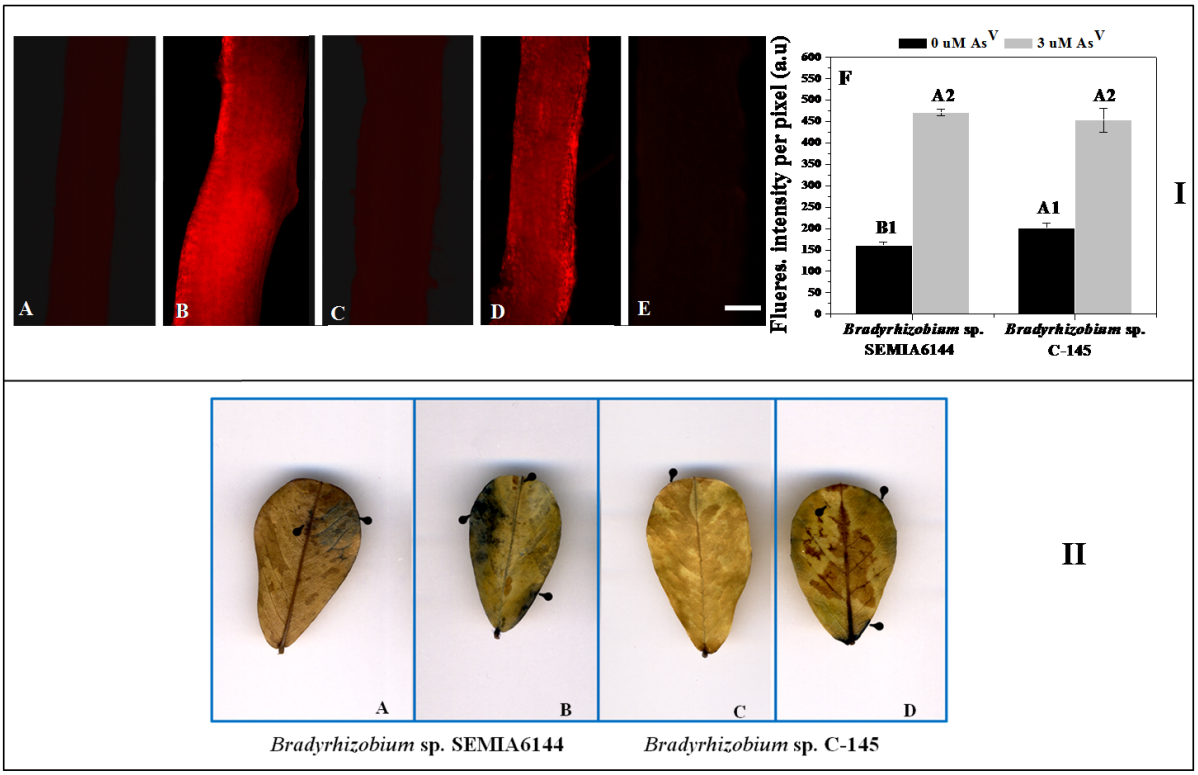
Ct: total chlorophyll, Ca: chlorophyll *a*, Cb: chlorophyll *b*

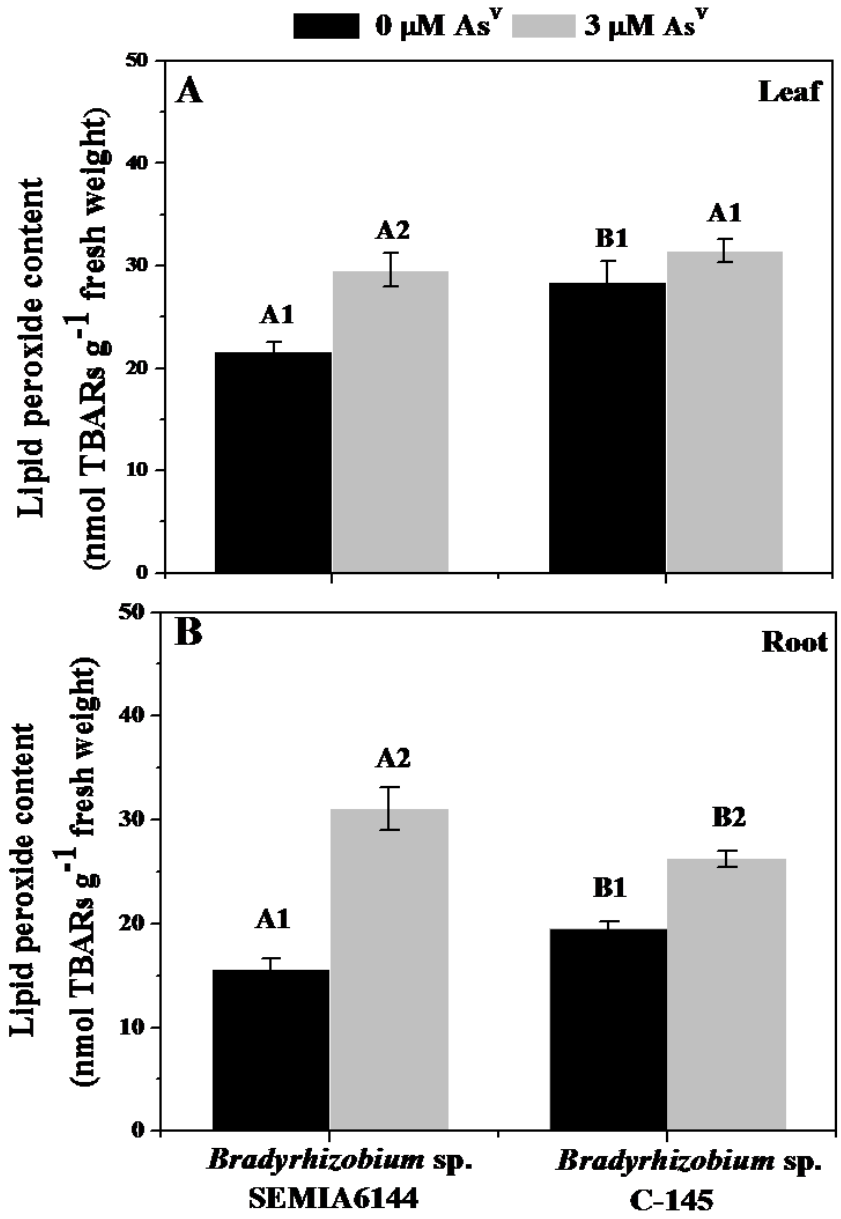


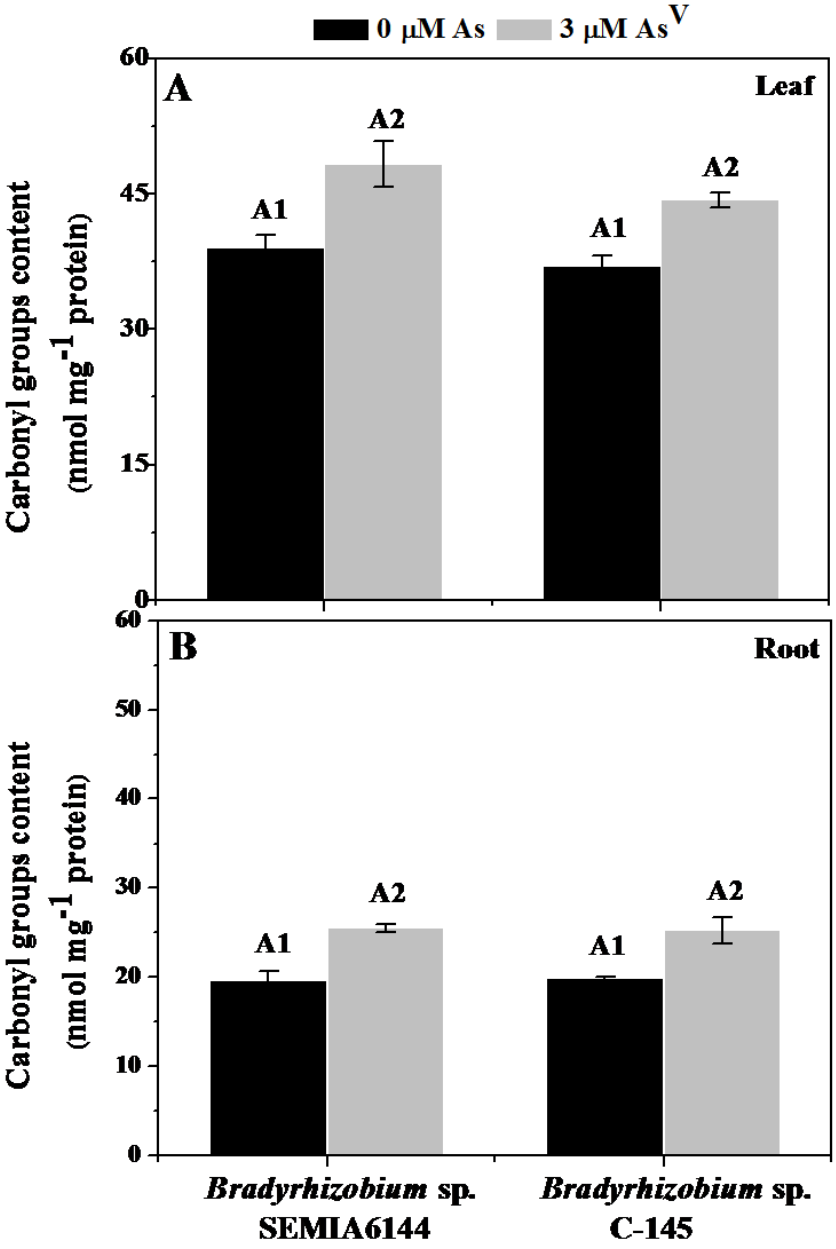
Bradyrhizobium **sp. SEMIA6144** ***Bradyrhizobium*** **sp. C-145**


0 μM As^V
3 μM As^V
A
Leaf
NADPH oxidase activity
(mmol NADP+ mg-1 protein min-1)
A1
A2
B1
A2
B
Root
NADPH oxidase activity
(mmol NADP+ mg-1 protein min-1)
A1
B2
B1
A2
Bradyrhizobium sp.
SEMIA6144
Bradyrhizobium sp.
C-145


I
A
B
C
D
E
II
A
B
C
D
Bradyrhizobium sp. SEMIA6144
Bradyrhizobium sp. C-145
III
Roots H_2O_2 content
(nmol H_2O_2 g^{-1} fresh weight)
0 μM As^V
3 μM As^V
A1
A2
B1
Bradyrhizobium sp. SEMIA6144
Bradyrhizobium sp. C-145
IV
Leaves H_2O_2 content
(nmol H_2O_2 g^{-1} fresh weight)
0 μM As
3 μM As^V
A1
A2
B1
B2
Bradyrhizobium sp. SEMIA6144
Bradyrhizobium sp. C-145


A
B
C
D
E
Flueres. intensity per pixel (a.u)
F
0 uM As V
3 uM As V
A2
A2
B1
A1
Bradyrhizobium sp. SEMIA6144
Bradyrhizobium sp. C-145
I
A
B
C
D
II
Bradyrhizobium sp. SEMIA6144
Bradyrhizobium sp. C-145


0 µM As^V
3 µM As^V
A
Leaf
Lipid peroxide content
(nmol TBARs g-1 fresh weight)
A1
A2
B1
A1
B
Root
A1
A2
B1
B2
Bradyrhizobium sp.
SEMIA6144
Bradyrhizobium sp.
C-145


0 μM As
3 μM As^{V}
A
Leaf
B
Root
Carbonyl groups content
(nmol mg^{-1} protein)
A1
A2
0
15
30
45
60
10
20
40
50
Bradyrhizobium sp.
SEMIA6144
Bradyrhizobium sp.
C-145

Highlights

- The redox response of the peanut-bradyrhizobia symbiosis exposed to As^{V} is unveiled.
- Root anatomy changes are the first defense mechanism against As entry.
- The NADPH activity in peanut organs is modulated by the rhizobial tolerance.
- Oxidative stress is *Bradyrhizobium* sp. strain-dependent and organ-differential.

Declaration of interests

X The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: